EXHIBIT 39

Nonisotopic Probing, Blotting, and Sequencing

SECOND EDITION

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Front cover photograph: Color enhanced digitized image of a DNA sequence obtained using the chemiluminescent substrate CSPD to visualize bound alkaline phosphatase conjugate. This illustration was kindly provided by Irena Bronstein and Chris Martin of Tropix, Inc.

This book is printed on acid-free paper. ♾

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9 Detection of Horseradish Peroxidase by Colorimetry

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I. INTRODUCTION

A. Historical Overview

Horseradish peroxidase (HRP) has been used extensively as a colorimetric marker in biological studies. A hemoprotein with a molecular weight of 40,000, HRP is an ideal detection reagent because of its stability, high turnover rate, and the availability of a wide assortment of colorimetric

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2. Sandwich Hybridizations

Sandwich assays are particularly well suited to peroxidase detection. These assays generally include a capture probe that is bound to a fixed matrix, such as nitrocellulose membranes (Dunn and Hassell, 1977; Ranki *et al.*, 1983), microtiter wells (Dahlen *et al.*, 1987; Keller *et al.*, 1989), or beads (Langdale and Malcolm, 1985; Polsky-Cynkin *et al.*, 1985). The target nucleic acid molecule hybridizes to the capture probe and is thereby bound to the matrix, while a second probe that is either directly or indirectly labeled with peroxidase hybridizes to an adjacent sequence on the target (Fig. 2). These techniques are particularly useful for the detection of polymerase chain reaction (PCR)-amplified nucleic acids, and have been used in assays for human immunodeficiency virus (HIV) (Kemp *et al.*, 1990; Keller *et al.*, 1989), β-thalassemia (Saiki *et al.*, 1988, 1989), and sickle-cell anemia (Saiki *et al.*, 1988) among others. Peroxidase detection is useful for these types of assays because of the high turnover rate of the enzyme, and because of the availability of a number of sensitive substrates for soluble assays.

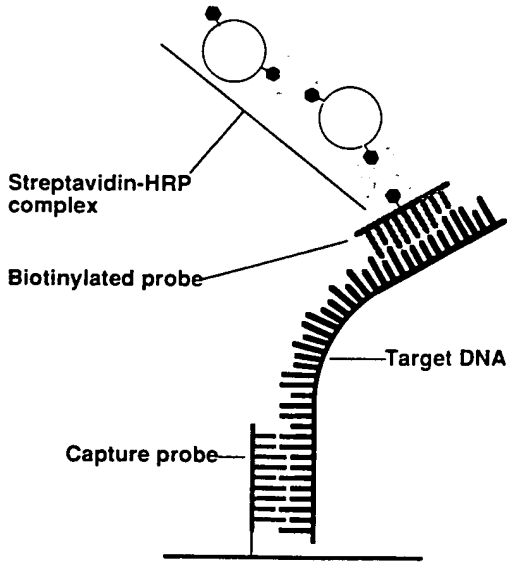

Fig. 2—Sandwich assays. The target hybridizes to an immobilized probe and is captured to the solid matrix. A biotinylated probe hybridizes to adjacent sequences on the target and is detected by streptavidin–HRP.